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MECHANICAL STIMULATION OF SKELETAL MUSCLE MITIGATES GLUCOCORTICOID-INDUCED DECREASES IN PROSTAGLANDIN SYNTHESIS

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ABSTRACT

The glucocorticoid dexamethasone (Dex) induces a decline in protein synthesis and protein content of tissue cultured, avian skeletal muscle cells, and this atrophy is attenuated by repetitive mechanical stretch. Since the prostaglandin synthesis inhibitor indomethacin mitigated this stretch attenuation of muscle atrophy, the role of prostaglandins as growth modulators in these processes was examined. Dex at 10^{-8}M reduced $PGF_{2\alpha}$ production 55%-65% and PGE_2 production 84-90% after 24-72 h of incubation in static cultures. Repetitive 10% stretch-relaxations of the non-Dex treated cultures increased $PGF_{2\alpha}$ efflux 41% at 24 h and 276% at 72 h, and increased PGE_2 production 51% at 24 h and 236% at 72 h. Mechanical stimulation of Dex treated cultures increased $PGF_{2\alpha}$ production 162% after 24 h, thus returning $PGF_{2\alpha}$ efflux to the level of non-Dex treated cultures. At 72 h, stretch increased $PGF_{2\alpha}$ efflux 65% in Dex treated cultures, but $PGF_{2\alpha}$ production was 45-84% less than non-Dex treated cultures. Mechanical stimulation of Dex treated cultures increased PGE_2 production at 24 h, but not at 72 h. Dex reduced prostaglandin H synthase (PGHS) activity in the muscle cultures by 70% after 8-24 h of incubation, and mechanical stimulation increased PGHS activity of the Dex treated cultures by 98%. It is concluded that repetitive mechanical stimulation attenuates the catabolic effects of Dex on cultured skeletal muscle cells in part by reversing the Dex-induced declines in PGHS activity and prostaglandin production.

Key Words: Cyclooxygenase, dexamethasone, exercise, mechanical stretch, prostaglandin H synthase

INTRODUCTION

Glucocorticoid-induced skeletal muscle atrophy is attenuated by exercise *in vivo* (3, 9) and repetitive mechanical stretch of tissue cultured, avian skeletal myofibers *in vitro* (2). *In vivo,* treadmill running decreases the myosin heavy chain degradation rate in glucocorticoid treated rats (3), and partially reverses Dex-induced increases in myofibrillar protease activity (20). Intermittent passive stretch of organ cultured skeletal muscle mitigates glucocorticoid-induced decreases in total protein synthesis rates (16). In tissue culture, repetitive mechanical stretch of the skeletal myofibers attenuates the atrophy induced by the synthetic glucocorticoid, dexamethasone (Dex), by partially reversing Dex-induced decreases in total protein synthesis and myosin heavy chain synthesis rates (2). The mechanism by which glucocorticoids and mechanical activity interact either *in vivo* or *in vitro* to regulate skeletal muscle protein turnover is not known.

The first purpose of this study was to examine whether attenuation of Dex-induced muscle atrophy by mechanical stretch involves alterations in $PGF_{2\alpha}$ and PGE_2 production. Prostaglandins are autocrine/paracrine growth factors in many cell types whose synthesis is inhibited by glucocorticoids (5). In skeletal muscle, prostaglandins are mechanogenic second messengers that regulate skeletal muscle protein turnover in response to mechanical activity/tension both *in vivo* and *in vitro* (15, 27). Increased $PGF_{2\alpha}$ and PGE_2 production occurs in response to muscle contraction *in vivo* (22), and mechanical stretch of both organ cultured and tissue

cultured skeletal muscle (16, 27). Stretch-induced increases in the anabolic prostaglandin $PGF_{2\alpha}$ are closely associated with increased protein synthesis rates in organ cultured skeletal muscle (18, 19, 21) and tissue cultured skeletal muscle (27), while increases in the catabolic prostaglandin PGE_2 in organ cultured muscle and mechanically stimulated tissue cultured skeletal muscle are associated with elevated protein degradation rates (19, 27). Stretch-induced compensatory hypertrophy in rats, and the associated increases in protein synthesis and degradation rates, are decreased by fenbufen, a prostaglandin synthesis inhibitor, suggesting that mechanical tension regulates skeletal muscle protein turnover *in vivo* by altering prostaglandin production (13).

Prostaglandin H synthase (PGHS) determines net prostaglandin production by catalyzing the synthesis of prostaglandins from arachidonic acid (4). PGHS activity is reduced by glucocorticoids in various cell types (4, 5), and mechanical stretch of tissue cultured muscle cells activates this enzyme (31). The second purpose of this study was to examine the interaction of repetitive mechanical stimulation and Dex on PGHS activity in the tissue cultured skeletal muscle cells. Our results show that Dex decreases $PGF_{2\alpha}$ and PGE_2 efflux from tissue cultured skeletal muscle, and this decrease is associated with a reduced PGHS activity. Repetitive mechanical stimulation of the tissue cultured skeletal muscle cells partially reverses these Dex-induced alterations. The results indicate that repetitive mechanical stimulation *in vitro* attenuates the catabolic effects of Dex on skeletal muscle by reversing the Dex-induced declines in PGHS activity and $PGF_{2\alpha}$ production.

MATERIALS AND METHODS

Cell Cultures. Avian myoblasts were isolated from 12 days *in ovo* pectoralis muscle by standard dissection techniques (28). Fertilized chicken eggs were obtained from Beaver River Farm (West Kingston, RI). Cells were plated at a very high density of 7,900 cells/mm^2 in 0.5 ml of 85/10/5 medium, and maintained in a humidified 5.0% CO_2 incubator at 37°C. 85/10/5 medium is Eagle's basal medium containing 10% horse serum (Sigma Chemical Co., St. Louis, MO), 5% chicken embryo extract, and 50 U/ml penicillin G (Sigma Chemical Co., Cat. No. P3414). The tissue culture plates and the wells of the mechanical cell stimulator were coated with type I collagen (Collaborative Research, Bedford, MA) prior to plating the cells. Cells were grown in collagen-coated 15 mm diameter tissue culture plates (Nunc Corp., Roskilde, Denmark) or in the collagen-coated 15 mm diameter culture wells of the mechanical cell stimulator. At 48-54 h postplating, the cells were embedded in a type I collagen gel matrix (28). Stainless steel wire cloth (mesh no. 50, Newark Wire Cloth, Newark, NJ) was placed inside the perimeter of each well of the mechanical cell stimulator. The screen acts as an "artificial tendon" by providing a surface to which the differentiating cells and collagen gel can attach and be held under tension during long-term growth in defined medium. Fresh medium was added to the top of the collagen gel 24 h after solidifying and changed every 24 h thereafter. After several days, the collagen gel dehydrated, collapsing on top of the muscle cells as a thin 12-50 μm

layer (28). Under these conditions, the myogenic cells proliferated and fused during the first 24 to 72 h *in vitro*, became striated and highly contractile by 96 to 120 h, and remained so for the duration of the experiments. By day 7-8 postplating, the cultures consisted of neonatal-like myofibers, unfused myoblasts, and interstitial fibroblasts. Approximately, 40-50% of the total culture nuclei were in unfused myoblasts or interstitial fibroblasts. Eighty-five to ninety percent of the noncollagenous protein in the cultures was located in the much larger multinucleated skeletal myofibers based on morphometric analysis of cell volumes (26).

On Day 7-8 postplating, the cultures were rinsed over a 2 h period with Eagle's basal medium containing 50 U/ml penicillin (0.5 ml/well, 5 x 20 min changes) on a rotary shaker (60 rpm) at 37°C. Serum-free muscle maintenance medium (MM medium) (29) without supplements or containing 10^{-8}M Dex (Sigma Chemical Co., Cat. No. D4902) was added to the cultures (500 µl/well). Dex was solubilized as a stock solution [10^{-2}M] in 100% ethanol. Final ethanol concentration in the medium was 0.0001%, and was also included in the control medium. MM medium consists of Eagle's basal medium containing 1,250 mg/l bovine serum albumin (Sigma Chemical, Cat. No. A4161), 0.05 mg/l sodium selenite (Sigma Chemical, Cat. No. S0882), 0.835 mg/l ferrous sulfate (Sigma Chemical, Cat. No. F8633), and 50 U/ml penicillin. All reagents were cell culture tested by Sigma. The medium was changed every 24 h for the duration of each experiment.

Mechanical Stimulation. The mechanical cell stimulator used to stretch and relax the culture substratum and the attached muscle cells has been described previously (25). Briefly, a cell growth chamber containing 24 15 mm diameter wells was secured above a

movable aluminum platform containing 2 mm wide by 11 mm high aluminum prongs, which center on the culture wells. For each experiment, the number of culture wells that were mechanically stimulated was adjusted by adding or removing the aluminum prongs from the movable platform. A stepper motor moved the prong platform up and down in 35 μm increments causing the prongs to stretch and relax the elastic substratum (0.01" thick Silastic™, Dow Corning Corp., Midland, MI) and attached cells. The activity pattern of the mechanical cell stimulator was controlled by an Apple IIe computer connected to the stepper motor. Mechanical activity program TRIAL82.PGM (Fig. 1), which has been shown to attenuate Dex-induced skeletal muscle atrophy (2), was used to repetitively stretch and relax the substratum on which the cells grew. This program consisted of five 10% substratum stretches and relaxations during a 20 s period followed by a 10 s rest; this activity pattern was repeated two more times followed by a 5 min rest period. The cells were mechanically stimulated for a total of 60 s every 6 min 20 s, i.e. 16% of experiment time. The rate of stretch and relaxation was 1.75 mm/s. Unstretched controls were maintained in the same growth chamber as the mechanically stimulated cells. The cell growth growth chamber was maintained on a rotary shaker (50-60 rpm) during the period of mechanical stimulation to equalize medium stirring differences between groups. Mechanical stimulation was started on day 7-8 postplating when the myofibers were well differentiated and structurally strong enough to withstand repetitive mechanical forces.

Fig. 1

Biochemical Assays. Total protein synthesis was measured as L-[U-^{14}C]phenylalanine (Amersham, Arlington Heights, IL) (1.0 μCi/ml)

incorporated into trichloroacetic acid (TCA)-precipitable material during a 4 h incubation period in the presence of 0.6 mM nonradioactive phenylalanine as previously outlined (26). Incorporation of [^{14}C]phenylalanine was linear during this time period. After incubation the cultures were detached from the wells and rinsed with ice-cold Earle's balanced salt solution (1 ml/well, 5 x 10 min changes) on a rotary shaker (120 rpm). The rinsed cells were sonicated in 0.5 ml ice-cold sucrose buffer (0.25 M sucrose, 0.02 M KCl, pH 6.8) and aliquots removed for determination of total noncollagenous protein, and trichloroacetic (TCA) acid soluble and precipitable radioactivity. Noncollagenous proteins were extracted by the method of Lowry *et al.* (11), and protein content was determined by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). For the protein synthesis assay, an aliquot of the sonicate was made 5% (vol/vol) with ice-cold TCA. After 30 min at 4°C, the sonicate was centrifuged at 3,000 *g* at 4°C for 10 min. The TCA soluble radioactivity in the supernatant was measured with a Packard 460C scintillation counter. The precipitate was rinsed three times with 5% TCA (vol:vol), the pellet was dissolved in 0.1 N NaOH, and TCA precipitable radioactivity was measured. Protein synthesis rates were expressed as [^{14}C]phenylalanine TCA precipitable dpms per μg total noncollagenous protein.

For determination of $PGF_{2\alpha}$ and PGE_2 production, serum-free MM medium was collected every 24 hours over the course of the experiment, and an aliquot used to measure $PGF_{2\alpha}$ or PGE_2 content by enzyme immunoassay (EIA; Cayman Chemical Co., Ann Arbor, MI). Sensitivity of the $PGF_{2\alpha}$ EIA at 22°C and 80% binding (B)/inital binding (B_0) was 24 pg/ml. The cross-reactivity of the $PGF_{2\alpha}$ antibody

was 100% for $PGF_{1\alpha}$, 7.0% for PGD_2, 2.0% for 6-keto $PGF_{1\alpha}$, 0.3% for 2,3-dinor-6-keto $PGF_{1\alpha}$ and <0.1% for all other eicosanoids including PGE_2. Sensitivity of the PGE_2 EIA at 22°C and 80%B/B_0 was 7 pg/ml. Cross-reactivity of the PGE_2 antibody was 9.2% for 15-keto PGE_2, 5.0% for PGE_1, and <0.1% for all other eicosanoids including $PGF_{2\alpha}$. Intraassay and interassay coefficients of variation for both assays were $\leq$ 10%.

PGHS activity was determined by removing the culture media at the indicated times, and adding fresh MM medium $\pm$ 10^{-8}M Dex supplemented with a saturating amount of arachidonic acid (30 μM) to the cells. After 30 min incubation, the medium was collected and an aliquot used to measure $PGF_{2\alpha}$ production by EIA. PGHS activity was expressed as picograms of $PGF_{2\alpha}$ produced per mg noncollagenous protein per minute. Similar results were obtained whether $PGF_{2\alpha}$ or PGE_2 was used to calculate PGHS activity (data not shown). This method has been shown to be an accurate assay of *in situ* PGHS enzymatic activity in skeletal muscle cultures (31).

Data Analysis. All experiments were repeated at least twice with separate preparations of primary cells. Statistical analysis of the data within individual experiments were performed by t-tests for unpaired values (PC Statistician Software, Human Systems Dynamics, Northridge, CA).

RESULTS

Dex at 10^{-8}M reduced total noncollagenous protein content of the skeletal muscle cultures by 30% and total protein synthesis rate by 61% after 3 days in serum-free MM medium (Fig. 2). In the control cultures, the prostaglandin synthesis inhibitor, indomethacin (100 μM), induced a small but significant decreases in the protein synthesis rate. However, indomethacin did not affect the Dex-induced decreases in total protein content and protein synthesis rate. Repetitive 10% stretches of the Dex treated cultures for 3 days increased total protein content by 66% and protein synthesis rate by 59% compared to static, Dex treated cultures (Fig. 3) as previously found (2). Indomethacin (100 μM) significantly reduced the stretch-induced increases in protein content and protein synthesis rate. Thus, 54% and 49% of the stretch-induced attenuation of the Dex-induced decreases in total protein content and total protein synthesis rate were prevented by indomethacin, respectively (Fig. 3).

Fig. 2

Fig. 3

Mechanical stimulation of control cultures increased $PGF_{2\alpha}$ production 41% compared to static cultures over the 0-24 h period (Fig. 4A) and 276% for the 48-72 h period (Fig. 4B). In static muscle cultures, 10^{-8}M Dex decreased $PGF_{2\alpha}$ production by 55% at 0-24 h and 65% at 48-72 h (Fig. 4). Mechanical stimulation of Dex treated cultures increased $PGF_{2\alpha}$ efflux 162% at 0-24 h compared to static, Dex treated cultures, so that $PGF_{2\alpha}$ production was not significantly different from non-Dex treated cultures (Fig 4A). In the longer-term cultures, stretch was not as effective in reversing the Dex-induced decrease in $PGF_{2\alpha}$ production. Although, mechanical stimulation of the

Fig. 4

Dex treated cultures increased $PGF_{2\alpha}$ production 65%, only 35% of the Dex-induced decrease in $PGF_{2\alpha}$ production at 48-72 h was prevented by mechanical stimulation (Fig. 4B).

Mechanical stimulation of the control cultures also increased PGE_2 synthesis 51% at 0-24 h ($p=.069$; Fig. 5A) and 236% at 48-72 h ($p<.01$; Fig. 5B). PGE_2 production was decreased 84% and 90% in static cultures by 10^{-8}M Dex over the 0-24 h and 48-72 h periods, respectively (Fig. 5). Mechanical stimulation of the Dex treated cultures increased PGE_2 production 362% compared to static, Dex treated cultures at 0-24 h (Fig, 5A), but did not significantly increase PGE_2 production at 48-72 h (Fig 5B).

Fig 5

PGHS activity was assayed in day 7 skeletal muscle cultures as the cells were switched from a serum-containing medium to serum-free MM medium. Enzymatic activity decreased by 57% during the 2 h rinse period in unsupplemented Eagle's Basal Medium at 37°C (Table 1). When these cultures were incubated in the serum-free MM medium, PGHS activity increased over the next 4-8 h, and this increase was maintained for up to 24 h (Table 2). 10^{-8}M Dex prevented this increase in PGHS activity from occurring in these static cultures (Table 2). PGHS activity was 36% less in the Dex treated cultures compared to non-Dex treated controls after 4 h and 70% less at 8-24 h.

Table 1

Table 2

Mechanical stimulation of the control cultures did not significantly increase PGHS activity at 24 h (Fig 6), but in Dex treated cultures, mechanical stimulation increased PGHS activity by 98% compared to static, Dex treated cultures (Fig. 6). PGHS activity was still 45% to 65% less than static or mechanically stimulated cultures not treated with Dex.

Fig 6

DISCUSSION

In this paper, we show that the glucocorticoid dexamethasone, which causes rapid skeletal muscle atrophy, induces significant decreases in $PGF_{2\alpha}$ and PGE_2 production in tissue cultured skeletal muscle and is associated with a decline in PGHS activity. Repetitive mechanical stimulation of the tissue cultured cells partially reverses the Dex-induced decreases in protein content and protein synthesis, prostaglandin production, and PGHS activity. These results are consistent with the hypothesis that Dex-induced skeletal muscle atrophy, and mechanical stretch/exercise-induced attenuation of this atrophy, involve alterations in prostaglandin synthesis.

Although the mechanism by which prostaglandins stimulate cell growth are unknown, there are several possibilities. Exogenous $PGF_{2\alpha}$ and PGE_2 at concentrations similar to those released into the medium [10^{-11}M to 10^{-9}M] by static and mechanically stimulated cultures do not alter protein turnover rates in cultured skeletal muscle (27). Thus, the prostaglandins may act synergistically with additional signalling systems to regulate protein turnover (6, 7, 32). For example, $PGF_{2\alpha}$ potentiated the proliferation response of cultured osteoblast to insulin-like growth factor-1 (IGF-1) (7). $PGF_{2\alpha}$ has also been shown to modulate IGF-1 receptor numbers (7). Thus prostaglandins may act synergistically with IGF-1 or other growth factors to stimulate cell growth.

Our results are consistent with $PGF_{2\alpha}$ being an anabolic autocrine/paracrine growth regulator in skeletal muscle, and that both glucocorticoids (18) and mechanical tension/activity (15, 16, 27) regulate skeletal muscle protein synthesis rates in part by

altering $PGF_{2\alpha}$ production. First, changes in $PGF_{2\alpha}$ production induced by Dex and mechanical stretch parallel the effects of Dex and stretch on protein synthesis rates (2). This is consistent with the Dex-induced decrease in $PGF_{2\alpha}$ release and protein synthesis in organ cultured skeletal muscle, and their partial reversal by intermittent stretching (18). Secondly, exogenous $PGF_{2\alpha}$ stimulates protein synthesis rates in tissue cultured skeletal muscle (27) and organ cultured skeletal muscle (21). Finally, the prostaglandin synthesis inhibitor, indomethacin, prevents approximately 50% of the stretch attenuation of the Dex-induced decline in protein synthesis rate in the cultured muscle cells. Indomethacin has little or no effect on basal levels of prostaglandin production (16, 27) and protein synthesis rates (16, 21), but completely inhibits the stretch-induced increases in $PGF_{2\alpha}$ production in these cells (27). The slight decrease in protein synthesis in the unstretched, indomethacin treated cells in this study (Fig. 2B) may be due to indomethacin blocking an increase in protein synthesis induced by addition of fresh culture media. Indomethacin also reduces stretch-induced increases in prostaglandin production and protein synthesis rates in organ cultured skeletal muscle (16, 21). *In vivo*, the prostaglandin synthesis inhibitor, fenbufen, reduces the elevated protein synthesis rates resulting from stretch-induced compensatory hypertrophy (13), and indomethacin retards the growth of skeletal muscle recovering from hindlimb suspension (23). Thus, $PGF_{2\alpha}$ appears to be an important mediator of Dex and mechanical stretch induced changes in skeletal muscle protein synthesis rates. Since indomethacin does not completely reverse the attenuation by mechanical stretch of the Dex-induced decrease in protein synthesis

rate, mechanical stretch probably stimulates protein synthesis by other signalling mechanisms in addition to $PGF_{2\alpha}$.

PGE_2 regulates protein degradation rates in skeletal muscle under certain experimental conditions. Exogenous PGE_2 increases protein degradation rates in organ cultured, rat skeletal muscle (19), and in tissue cultured, avian skeletal muscle (27). Under other experimental conditions, PGE_2 does not affect protein degradation rates in organ cultured, rat skeletal muscle (1, 8, 24) and in tissue cultured, avian and rat skeletal muscle (12). In the present study, PGE_2 production is inhibited by Dex, and mechanical stimulation elevated PGE_2 production at 0-24 h but not at 48-72 h. The relationship of these alterations to stretch attenuation of Dex-induced muscle atrophy is unclear. Glucocorticoid-induced increases in protein degradation have been reported in vivo (14, 17), but the relatively small changes are considered less important for the loss of muscle protein than the larger decreases in protein synthesis rates. Since glucocorticoids decrease PGE_2 production in this and other studies, any Dex-induced increase in protein degradation must result from mechanisms other than PGE_2-associated signalling pathways. Since the primary mechanism for glucocorticoid induced muscle atrophy is a decreased protein synthesis rate, changes in protein degradation and PGE_2 production may be of minor importance for the sparing of muscle protein compared to the stretch-induced attenuation of Dex-induced declines in $PGF_{2\alpha}$ production.

Fibroblasts are an essential element of this *in vitro* model system since they synthesize extracellular matrix components necessary for the muscle cells to withstand repetitive mechanical stimulation without damage. This mixed myofiber-fibroblast

population is similar to the *in vivo* arrangement of myofibers and fibroblasts. Either cell type may release prostaglandins into the extracellular space that can act on the same or other cell types. The cell type in which Dex alters prostaglandin production is not known, but altered prostaglandin production in fibroblasts could affect myofiber growth since prostaglandins are paracrine growth regulators. All of the stretch-induced increase in PGE_2 production and 90% of the stretch-induced $PGF_{2\alpha}$ production appears to be from the skeletal myofibers (31), suggesting that prostaglandins may act as autocrine growth regulators.

PGHS activity decreases when the cultures are rinsed with unsupplemented basal medium (Table 1), but increases when these cultures are exposed to MM medium (Table 2). Apparently, a component of the MM medium induces an increase in PGHS activity. Addition of serum-containing medium induces a 2.5-fold increase in PGHS activity after 6 h (data not shown), indicating a strong, serum-inducible enzyme activity in these cultures. Dex suppresses the increase in PGHS activity induced by the addition of MM medium. These results are consistent with the rapid serum-induced increase in the mitogen-inducible PGHS protein isoform (TIS10/PGS-2) in cultured fibroblasts, which is supressed by Dex (10). In our studies, repetitive 10% stretches increase PGHS activity in the Dex treated cultures. Thus, mechanical stretch mitigates the Dex-induced decrease in prostaglandin production by activiating PGHS. Whether stretch affects the activity of the constitutive or mitogen-inducible isoforms is not known.

In addition to prostaglandin production being determined by PGHS activity, prostaglandin synthesis is also regulated by the

availability of arachidonic acid, which results from the activity of several phospholipases (4). It has been suggested that the inhibitory action of Dex on muscle protein synthesis results from a decline in the activity of phospholipase A_2 (18). It has been shown that Dex (5) and mechanical stretch (30) affect phospholipase A_2 activity. Thus, changes in prostaglandin synthesis induced by Dex and mechanical stretch may also be due to mechanisms other than changes in PGHS activity.

The results of this study show for the first time that Dex induces a dramatic decrease in $PGF_{2\alpha}$ and PGE_2 production of tissue cultured avian skeletal muscle that is attenuated by repetitive mechanical stimulation. The changes in $PGF_{2\alpha}$ and PGE_2 production induced by Dex and mechanical stretch are due in part to parallel changes in PGHS activity. These results indicate that repetitive mechanical stimulation of tissue cultured skeletal muscle may attenuate the catabolic effects of Dex by partially reversing the Dex-induced declines in PGHS activity and $PGF_{2\alpha}$ synthesis.

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REFERENCES

1. **Barnett, J. G., and S. Ellis.** Prostaglandin E_2 and the regulation of protein degradation in skeletal muscle. *Muscle & Nerve 10*: 556-559, 1987.

2. **Chromiak, J. A., and H. H. Vandenburgh.** Glucocorticoid-induced skeletal muscle atrophy in vitro is attenuated by mechanical stimulation. *Am. J. Physiol. 262 (Cell Physiol. 31)*: C1471-C1477, 1992.

3. **Czerwinski, S. M., R. Zak, T. T. Kurowski, M. T. Falduto, and R. C. Hickson.** Myosin heavy chain turnover and glucocorticoid deterrence by exercise in muscle. *J. Appl. Physiol. 67*: 2311-2315, 1989.

4. **DeWitt, D. L.** Prostaglandin endoperoxide synthase: regulation of enzyme expression. *Biochim. Biophys. Acta 1083*: 121-134, 1991.

5. **Duval, D., and M. Freyss-Beguin.** Glucocorticoids and prostaglandin synthesis: we cannot see the wood for the trees. *Prostaglandins, Leuk. Essen. Fatty Acids 45*: 85-112, 1992.

6. **Goin, M., O. Pignataro, and L. J. de Asua.** Early cell cycle diacylglycerol (DAG) content and protein kinase C (PKC) activity enhancement potentiates prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induced mitogenesis in Swiss 3T3 cells. *FEBS Lett. 316*: 68-72, 1993.

7. **Hakeda, Y., S. Harada, T. Matsumoto, K. Tezuka, K. Higashino, H. Kodama, T. Hashimoto-Goto, E. Ogata, and M. Kumegawa.** Prostaglandin $F_{2\alpha}$ stimulates proliferation of clonal osteoblastic MC3R3-E1 cells by up-regulation of insulin-like growth factor I receptors. *J. Biol. Chem. 266*: 21044-21050, 1991.

8. **Hasselgren, P-O., Aamir, O., James, H., and J. E. Fischer.** Prostaglandin E_2 does not regulate total or myofibrillar protein breakdown in incubated skeletal muscle from normal or septic rats. *Biochem. J. 270*: 45-50, 1990.

9. **Hickson, R. C., and J. R. Davis.** Partial prevention of glucocorticoid-induced muscle atrophy by endurance training. *Am. J. Physiol. 241*: E226-E232, 1981.

10. **Kujubu, D. A., S. T. Reddy, B. S. Fletcher, and H. R. Herschman.** Expression of the protein product of the prostaglandin synthase-2/TIS10 gene in mitogen-stimulated Swiss 3T3 cells. *J. Biol. Chem. 268:* 5425-5430, 1993.

11. **Lowry, O. H., D. R. Gilligan, and E. Katersky.** The determination of collagen and elastin in tissues with results obtained in various normal tissues from different species. *J. Biol. Chem. 139*: 795-804, 1941.

12. **McElligot, M. A., L.-Y. Chaung, V. Baracos, and E. A. Gulve.** Prostaglandin production in myotube cultures. *Biochem. J. 253*: 745-749, 1988.

13. **McMillan, D. N., P. J. Reeds, G. E. Lobley, and R. M. Palmer.** Changes in protein turnover in hypertrophying plantaris muscles of rats: effect of fenbufen - an inhibitor of prostaglandin synthesis. *Prostaglandins 34*: 841-852.

14. **Odedra, B. R., and D. J. Millward.** Effect of corticosterone on muscle protein turnover in adrenalectomized rats and diabetic rats maintained on insulin. *Biochem. J. 204*: 663-672, 1982.

15. **Palmer, R. M.** Prostaglandins and the control of muscle protein synthesis and degradation. *Prostaglandins, Leuk. Essen. Fatty Acids. 39*: 95-104, 1990.

16. **Palmer, R. M., P. J. Reeds, T. Atkinson, and R. H. Smith.** The influence of changes in tension on protein synthesis and prostaglandin release in isolated rabbit muscles. *Biochem. J. 214*: 1011-1014, 1983.

17. **Rannels, S. R., and L. S. Jefferson.** Effects of glucocorticoids on muscle protein turnover in perfused rat hemicorpus. *Am. J. Physiol. 238 (Endocrinol. Metab. 1)*: E564-E572, 1980.

18. **Reeds, P. J., and R. M. Palmer.** Changes in prostaglandin release associated with inhibition of muscle protein synthesis by dexamethasone. *Biochem. J. 219*: 953-957, 1984.

19. **Rodemann, H. P., and A. L. Goldberg.** Arachidonic acid, Prostaglandin E_2 and $F_{2\alpha}$ influence rates of protein turnover in skeletal and cardiac muscle. *J. Biol. Chem. 257*: 1632-1638, 1982.

20. **Seene, T., and A. Viru.** The catabolic effect of glucocorticoids on different types of skeletal muscle fibres and its dependence upon muscle activity and interaction with anabolic steroids. *J. Steroid Biochem. 16*: 349-352, 1982.

21. **Smith, R. H., R. M. Palmer, and P. J. Reeds.** Protein synthesis in isolated rabbit forelimb muscles. *Biochem. J. 214*: 153-161, 1983.

22. **Symons, J. D., S. J. Theodossy, J. C. Longhurst, and C. L. Stebbins.** Intramuscular accumulation of prostaglandins during static contraction of the cat triceps surae. *J. Appl. Physiol. 71*: 1837-1842, 1991.

23. **Templeton, G. H., Padalino, M. and R. Moss.** Influences of inactivity and indomethacin on soleus phosphatidylethanolamine and size. *Prostaglandins 31*: 545- 559, 1986.

24. **Turinsky, J.** Phospholipids, prostaglandins E_2, and proteolysis in denervated muscle. *Am. J. Physiol. 251 (Regulatory Integrative Comp. Physiol. 20)*: R165-173, 1986.

25. **Vandenburgh, H. H.** A computerized mechanical cell stimulator for tissue culture: effects on skeletal muscle organogenesis. *In Vitro 24*: 609-619, 1988.

26. **Vandenburgh, H. H., S. Hatfaludy, P. Karlisch, and J. Shansky.** Skeletal muscle growth is stimulated by intermittent stretch-relaxation in tissue culture. *Am. J. Physiol. 256 (Cell Physiol. 25)*: C674-C682, 1989.

27. **Vandenburgh, H. H., S. Hatfaludy, I. Sohar, and J. Shansky.** Stretch-induced prostaglandins and protein turnover in cultured skeletal muscle. *Am. J. Physiol. 259 (Cell Physiol. 28)*: C232-C240, 1990.

28. **Vandenburgh, H. H., P. Karlisch, and L. Farr.** Maintenance of highly contractile tissue-cultured avian skeletal myotubes in collagen gel. *In Vitro, 24*: 166-174, 1988.

29. **Vandenburgh, H. H., P. Karlisch, J. Shansky, and R. Feldstein.** Insulin and IGF-I induce pronounced hypertrophy of skeletal myofibers in tissue culture. *Am. J. Physiol. 260 (Cell Physiol. 29)*: C475-C484, 1991.

30. **Vandenburgh, H. H., J. Shansky, P. Karlisch, and R. Lopez Solerssi.** Mechanical stimulation of skeletal muscle generates lipid-related second messengers by phospholipase activation. *J. Cell. Physiol. 155:* 63-71, 1993.

31. **Vandenburgh, H. H., J. Shansky, R. Solerssi, and J. Chromiak.** Mechnaical stimulation of skeletal muscle in vitro increases prostaglandin $F_{2\alpha}$ synthesis and cyclooxygenase by a pertussis toxin sensitive mechanism. Manuscript submitted for publication, 1993.

32. **Wahl, L. M., C. E. Olsen, A. L. Sandberg, and E. Mergenhagen.** Prostaglandin regulation of macrophage collagenase production. *Proc. Natl. Acad. Sci. USA 74*: 4955-4958, 1977.

TABLE 1. Prostaglandin H Synthase activity decreases when the cells are rinsed with unsupplemented Basal Medium Eagle's (BME).

TIME IN BME (h)	PGHS ACTIVITY ($PGF_{2\alpha}$: pg/mg protein/min)
0	66.7 ± 5.1
1	45.8 ± 4.8*
2	28.6 ± 4.1**

Muscle cultures were grown as described in MATERIAL AND METHODS. Day 7 cultures were rinsed briefly (less than 5 min; Time 0), or for 1 or 2 h with unsupplemented Basal Medium Eagle's. Immediately after rinsing, PGHS activity was determined as described in METHODS. Each point is the mean ± SE of 6 samples. Statistical analysis was by t-tests; *$p<.05$, **$p=.001$ vs. time 0 h.

TABLE 2. Dexamethasone [10^{-8}M] reduced Prostaglandin H Synthase activity in tissue cultured avian skeletal muscle.

	PGHS ACTIVITY ($PGF_{2\alpha}$: pg/mg protein/min)		
TIME (hr)	CONTROL	DEX	% Δ
2	26.60 ± 4.30	20.45 ± 2.52	-23.1%, $p > .05$
4	43.12 ± 3.36	27.80 ± 2.72	-35.5%, $p < .05$
8	121.76 ± 9.64	37.25 ± 2.72	-69.4%, $p = .001$
16	74.54 ± 6.19	22.60 ± 2.18	-69.7%, $p = .001$
24	63.24 ± 5.54	18.65 ± 2.13	-70.5%, $p = .001$

Day 7 muscle cultures were rinsed for 2 h with unsupplemented basal medium Eagle's, and placed in Muscle Maintenance Medium ± 10^{-8}M Dex. At each time point, PGHS activity was determined as described in METHODS. Each point is the mean ± SE of 4-5 samples; statistical analysis was by t-tests.

FIGURE LEGENDS

Figure 1. Schematic diagram of mechanical stretch-relaxation program (TRIAL82.PGM) initiated on Day 7-8 postplating. The elastic substratum and attached cells were stretched and relaxed 10% every 5 min. This activity pattern was continued for the duration of each experiment, and resulted in the cells being mechanically stimulated 16% of the experimental time period. Static controls were grown on the same elastic substratum in the same cell growth chamber, but were not mechanically stimulated.

Figure 2. Indomethacin does not prevent Dex-induced skeletal muscle atrophy in static cultures. Muscle cultures were grown in 4-well tissue culture plates as described in MATERIALS AND METHODS for 7 days, rinsed, and placed in MM Medium $\pm$ 10^{-8}M Dex $\pm$ 100 μM indomethacin for 3 days. Media were changed every 24 h. After 3 days, total noncollagenous protein content (A) and total protein synthesis rate (B) were measured. Each point is the mean $\pm$ SE of 4-6 samples. Statistical analysis was by t-tests: *$p<.05$ control, no indo vs. control + indo; **$p<.001$ control, no indo vs. Dex, no indo; ***$p<.001$ control + indo vs. Dex + indo.

Figure 3. Mechanical stretch attenuated Dex-induced skeletal muscle atrophy by an indomethacin sensitive mechanism. Muscle cultures were grown in the wells of the mechanical cell stimulator as described in MATERIALS AND METHODS for 7 days, rinsed, and placed in Muscle Maintenance Medium + 10^{-8}M Dex ± 100 μM indomethacin for 3 days. Media were changed every 24 h. Mechanical activity program TRIAL82.PGM was used to repetitively stretch and relax the elastic membrane and attached cells 10% (Fig. 1) for the duration of the experiment. After 3 days, total noncollagenous protein content (A) and total protein synthesis rate (B) were measured. Each point is the mean ± SE of 4-6 samples. Statistical analysis was by t-tests: *p<.01 Dex, no stretch vs. Dex + stretch; **p<.05 Dex, no stretch vs. Dex + stretch + indo; ***p<.01 Dex, no stretch vs. Dex + stretch + indo; +p<.05 Dex + stretch vs. Dex + stretch + indo.

Figure 4. Dex decreased $PGF_{2\alpha}$ production, and mechanical stimulation attenuated this decrease. Day 7-8 muscle cultures were grown as described in MATERIALS AND METHODS, rinsed, and placed in MM medium $\pm$ 10^{-8}M Dex. Half of the wells in each group were mechanically stimulated using TRIAL82.PGM. Media were collected every 24 h for determination of $PGF_{2\alpha}$ efflux and fresh media added. $PGF_{2\alpha}$ efflux for the 0-24 h (A) and 48-72 h (B) periods is shown. Each point is the mean $\pm$ SE of 3-5 samples. Statistical analysis was by t-tests: *$p \leq .01$ control, no stretch vs. control + stretch; **$p < .05$ control, no stretch vs. Dex, no stretch; ***$p = .001$ control, no stretch vs. Dex, no stretch; +$p < .001$ Dex, no stretch vs. Dex + stretch.

Figure 5. Dex decreased PGE_2 production, and mechanical stimulation attenuated this decrease at 0-24 h, but not at 48-72 h. Day 7-8 muscle cultures were grown as described in MATERIALS AND METHODS, rinsed, and placed in MM Medium ± 10^{-8}M Dex. Half of the wells in each group were mechanically stimulated using TRIAL82.PGM. Media were collected every 24 h for determination of PGE_2 efflux and fresh media added. PGE_2 production for the 0-24 h (A) and 48-72 h (B) periods is shown. Each point is the mean ± SE of 3-5 samples. Statistical analysis was by t-tests: *$p<.01$ control, no stretch vs. control + stretch; **$p\leq.001$ control, no stretch vs. Dex, no stretch; ***$p<.05$ Dex, no stretch vs. Dex + stretch.

Figure 6. Mechanical stimulation partially reversed the Dex-induced decrease in PGHS activity. Day 7 muscle cultures were rinsed and placed in MM Medium ± 10^{-8}M Dex. Half of the wells in each group were mechanically stimulated using TRIAL82.PGM. After 24 h, PGHS activity was determined as described in METHODS. Each point is the mean ± SE of 3-5 samples. Statistical analysis was by t-tests: *p<.01 control, no stretch vs. Dex, no stretch; **p<.05 Dex, no stretch vs. Dex + stretch.

Fig. 1

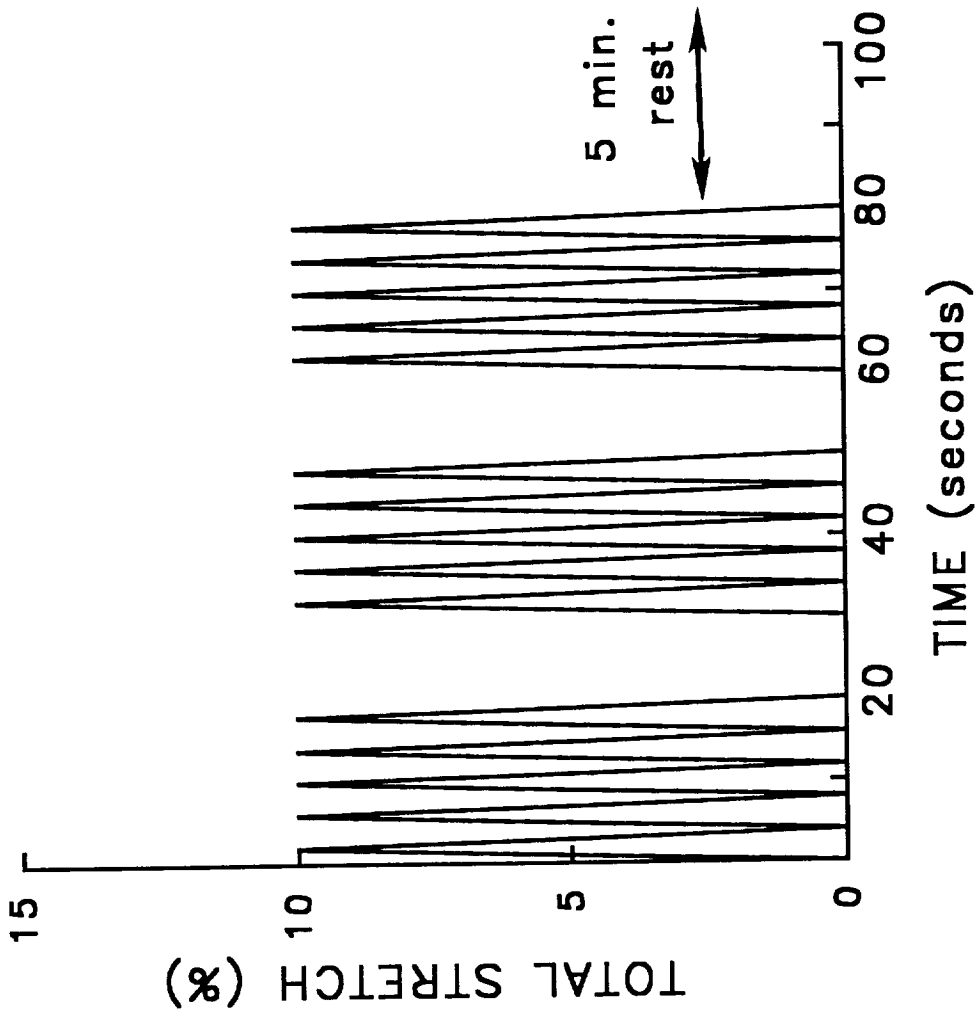


Fig. 2

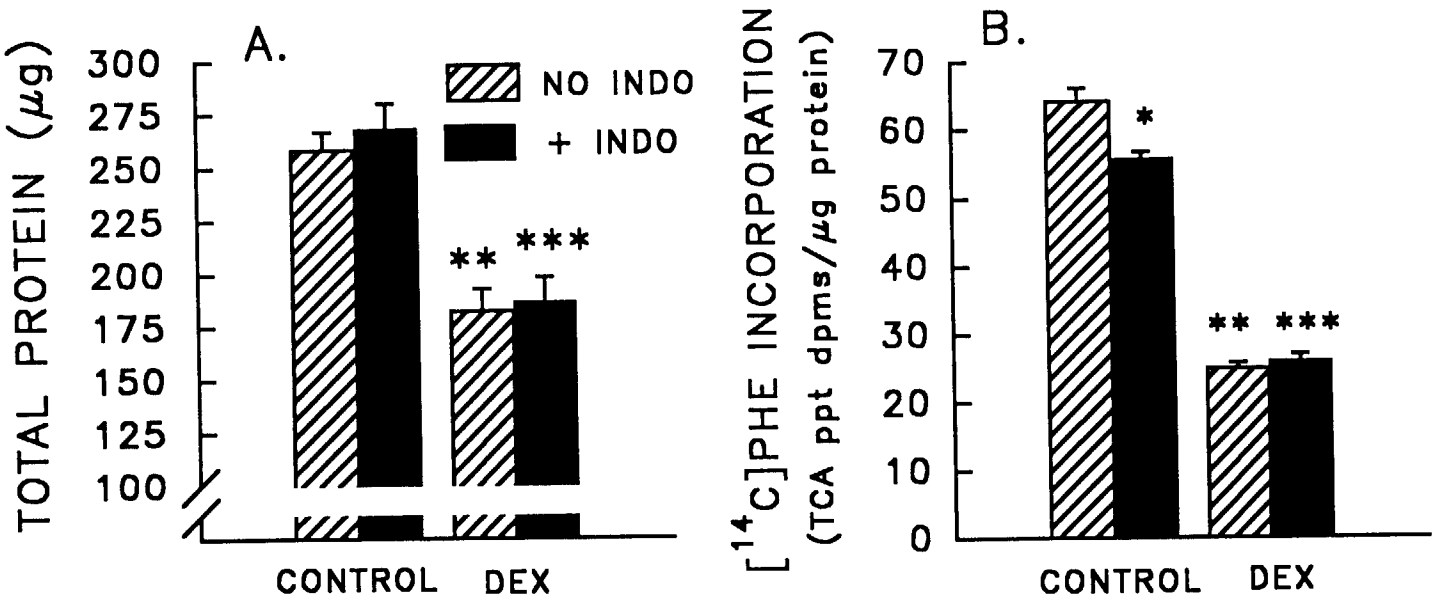

A.
TOTAL PROTEIN (μg)
300
275
250
225
200
175
150
125
100
NO INDO
+ INDO
**
***
CONTROL
DEX
B.
[14C]PHE INCORPORATION
(TCA ppt dpms/μg protein)
70
60
50
40
30
20
10
0
*
**
***
CONTROL
DEX

Fig. 3

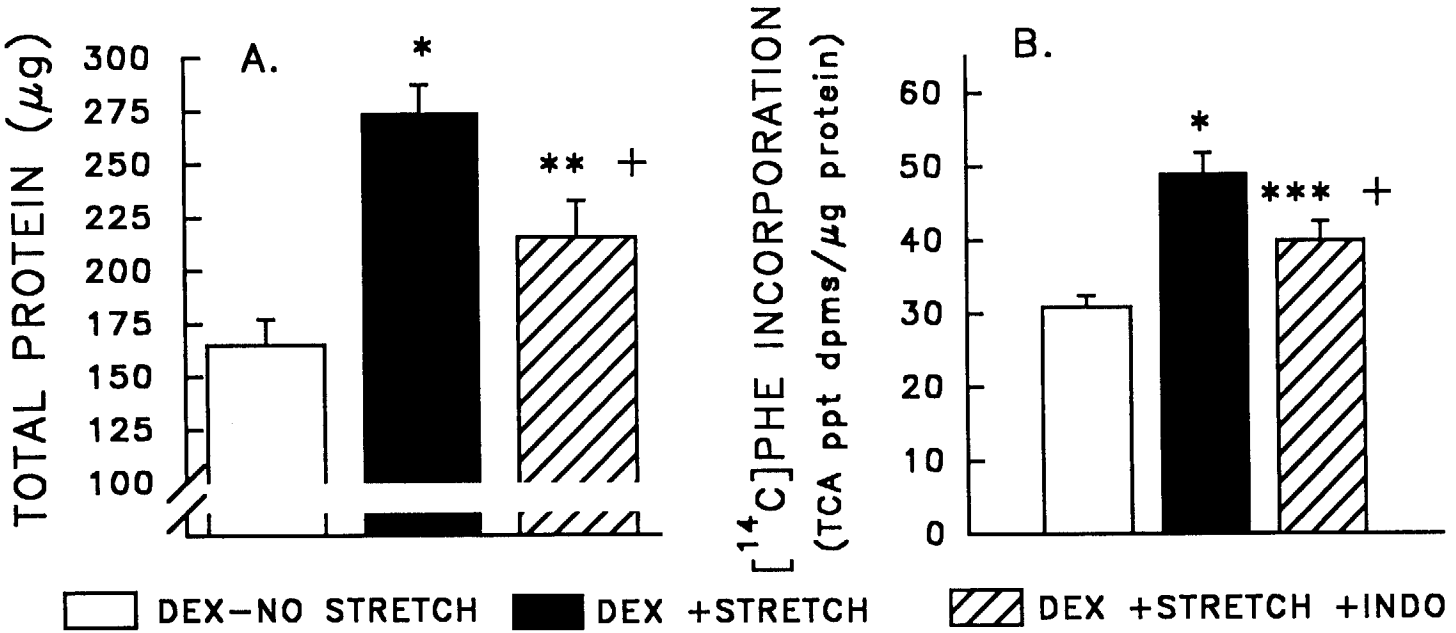

TOTAL PROTEIN (μg)
300
275
250
225
200
175
150
125
100
A.
*
** +
[14C]PHE INCORPORATION
(TCA ppt dpms/μg protein)
60
50
40
30
20
10
0
B.
*
*** +
DEX–NO STRETCH
DEX +STRETCH
DEX +STRETCH +INDO

Fig. 4

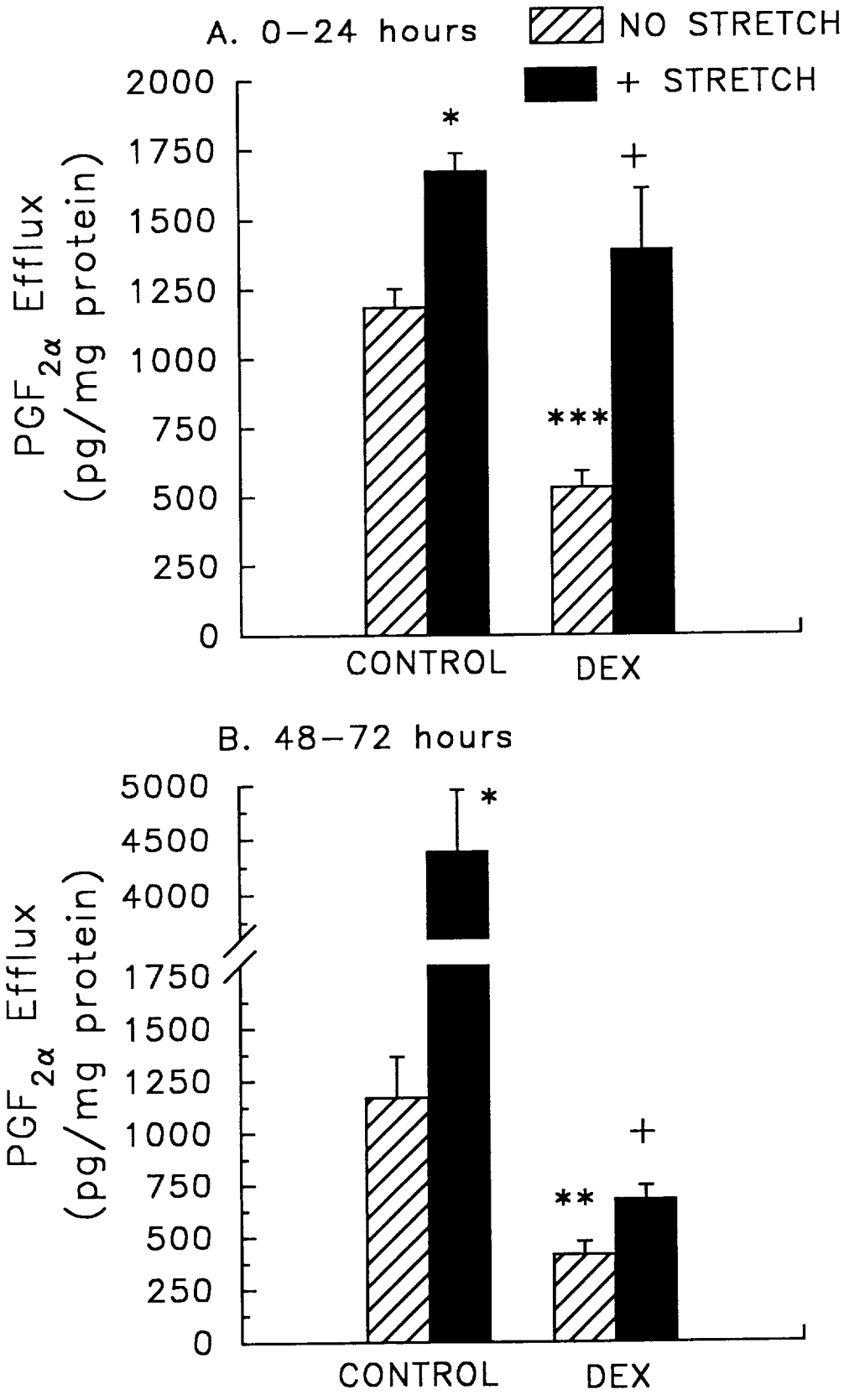

A. 0–24 hours
NO STRETCH
+ STRETCH
PGF2α Efflux (pg/mg protein)
2000
1750
1500
1250
1000
750
500
250
0
*
+
***
CONTROL
DEX
B. 48–72 hours
5000
4500
4000
1750
1500
1250
1000
750
500
250
0
*
+
**
CONTROL
DEX

Fig. 5

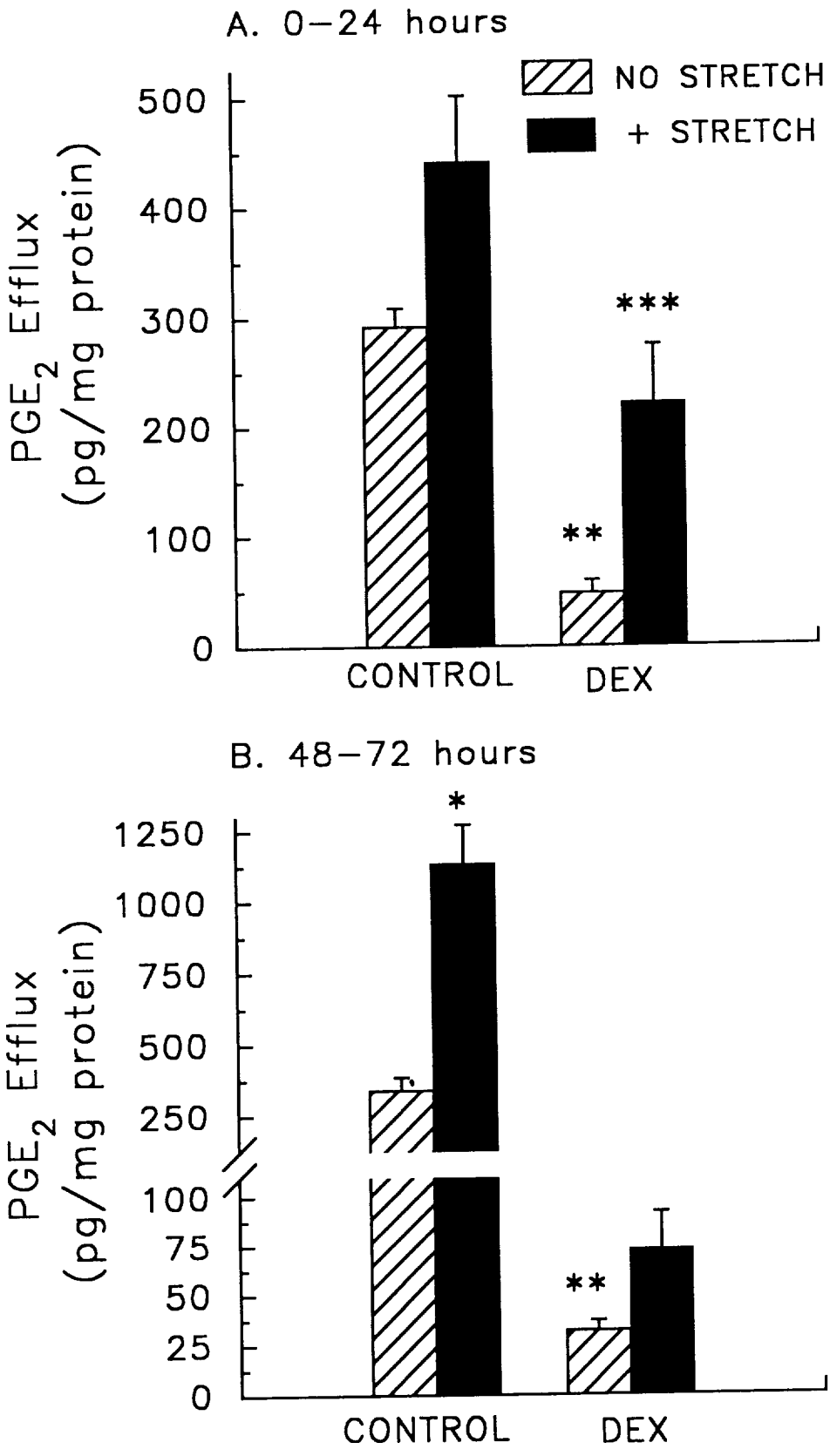
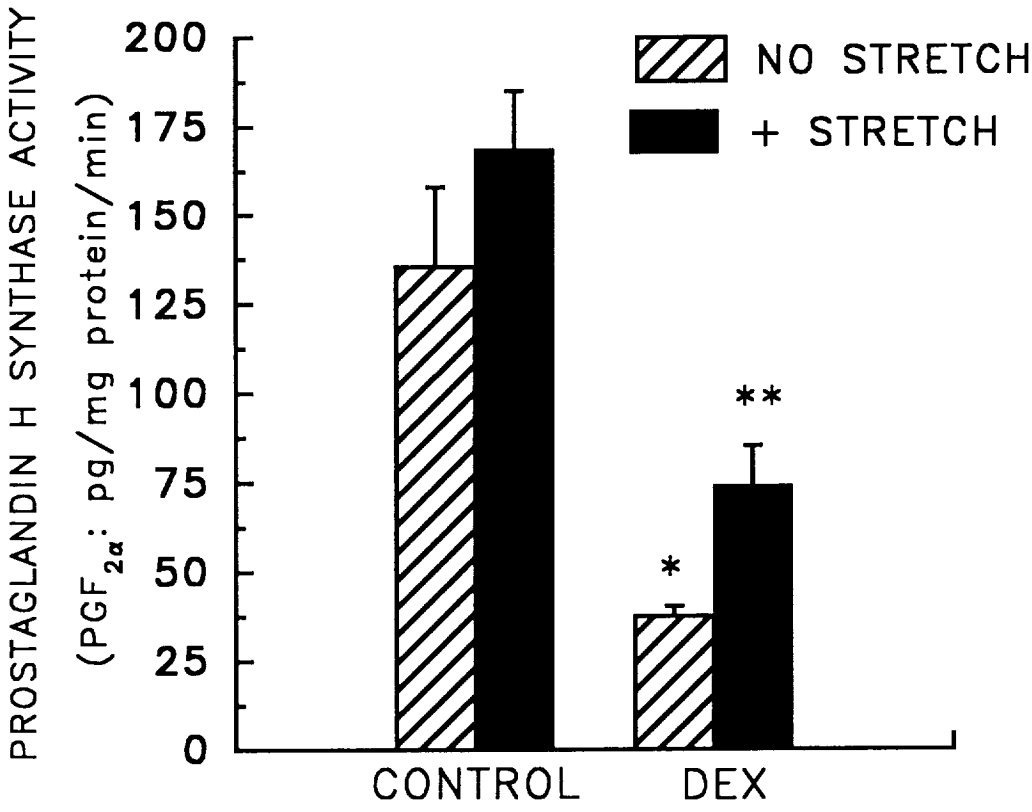

A. 0–24 hours
NO STRETCH
+ STRETCH
PGE2 Efflux
(pg/mg protein)
500
400
300
200
100
0
**
***
CONTROL
DEX
B. 48–72 hours
PGE2 Efflux
(pg/mg protein)
1250
1000
750
500
250
100
75
50
25
0
*
**
CONTROL
DEX

Fig. 6


PROSTAGLANDIN H SYNTHASE ACTIVITY
($PGF_{2\alpha}$: pg/mg protein/min)
200
175
150
125
100
75
50
25
0
NO STRETCH
+ STRETCH
*
**
CONTROL
DEX